DEVELOPMENT OF A SAMPLE ENRICHMENT PROBE METHOD FOR THE GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS OF HORMONES IN WATER

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ABSTRACT

A simple sample enrichment probe (SEP) sampling method was developed for the determination of endogenous steroid hormones (estrone, 17β-estradiol and testosterone) in water using gas chromatography-mass spectrometry (GC-MS). SEPs constructed of thin stainless steel rods of inert material or sections of a polyimide coated megabore GC column provided at one end with a short sleeve of polydimethylsiloxane (PDMS) rubber as the sorbent material were used. The selected target hormones were estrone, 17β-estradiol and testosterone. Extraction of the steroid hormones was performed by direct immersion of the SEP in water samples. To maximise the extraction efficiency of the SEP method to extract the analytes, several parameters affecting the extraction efficiency of the SEP method, such as extraction time, extraction temperature, pH and ionic strength were optimised. Due to challenges encountered with the GC-MS the method was only partially validated using ultra-pure water samples spiked with hormones at different concentration levels. Under the optimal conditions, the method showed satisfactory linearity over a concentration range of 1-10 μg/mL (for estrone and testosterone) and 2-10 μg/mL (for estradiol), with regression squares (R^2) ranging between 0.988-0.996. Acceptable reproducibility in the range of 2-17 % RSD was achieved. The obtained limits of quantitation (LOQs) were 0.5 μg/mL for the three steroids. Although the SEP-GC-MS method gave very high LOQs when compared to existing SPME and SBSE methods, it proved to be a simple and cost-effective alternative to these methods.

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LIST OF ABBREVIATIONS AND SYMBOLS

DART/OT-MS	Direct analysis in real time Orbitrap™ Mass Spectrometry
DI	Direct immersion
DLLME	Dispersive Liquid-Liquid Microextraction
DVB/CAR/PDMS	Divinylbenzene/Carboxen/polydimethylsiloxane
E1	Estrone
E2	17β-Estradiol
EDCs	Endocrine Disrupting Compounds
FPSE	Fabric Phase Sorptive Extraction
GC×GC-ToF/MS	Two Dimensional Gas Chromatography with Time of Flight Mass Spectrometry
GC-MS	Gas Chromatography–Mass Spectrometry
GC-MS/MS	Gas Chromatography with tandem Mass Spectrometry
GC-MS/MS (QqQ)	Gas Chromatography-triple Quadrupole Mass Spectrometry
GWWTP	Gammams Wastewater Treatment Plant
HPLC-DAD	High Performance Liquid Chromatography and Diode-array Detection
HPLC-FLD	High Performance Liquid Chromatography with Fluorescence Detection
HPLC/Q-TOF-MS	High Performance Liquid Chromatography coupled to Quadrupole-Time-of-Flight Mass Spectrometry

HPLC-UV	High Performance Liquid Chromatography with Ultraviolet detection
ICP-MS	Inductively Couple Plasma-Mass Spectrometry
HS	Headspace
IAC	Immunoaffinity Chromatography
IRMOF	Isoreticular Metal-organic Framework
LC-DAD	Liquid Chromatography and Diode-array Detection
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography with tandem Mass Spectrometry
LC-UV	Liquid Chromatography with Ultraviolet Diode-array Detection
LD	Liquid Desorption
LD-IC/CD	Liquid Desorption Ion Chromatography with Conductivity Detector
LDTD-APCI-MS/MS	Laser Diode Thermal Desorption/Atmospheric Chemical Ionisation tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
LVI-GC-FPD	Large Volume Injection Gas Chromatography-Flame Photometric Detection
MIL	Material of Institute Lavoisier
MIP	Molecularly Imprinted Polymer
MIT	Molecularly Imprinted Technology
MOF	Metal-organic Framework

OCPs	Organo-chlorine Pesticides
OGWRP	Old Goreangab Water Reclamation Plant
OPPs	Organophosphorus Pesticides
PA	Polyacrylate
PAHs	Polycyclic Aromatic Hydrocarbons
PDMS	Polydimethylsiloxane
POPs	Persistent Organic Pollutants
R^2	Regression Square
RAMs	Restricted Access Materials
RSD	Relative Standard Deviation
SBSE	Stir-bar Sorptive Extraction
SEP	Sample Enrichment Probe
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
SR	Silicone Rod
T	Testosterone
TD	Thermal Desorption
UHPLC	Ultra High Performance Liquid Chromatography
UHPLC-MS/MS	Ultra High Performance Liquid Chromatography tandem Mass Spectrometry
VOCs	Volatile Organic compounds
WINGOC	Windhoek Goreangab Operating Company
WRP	Water Reclamation Plant

WWTP	Wastewater Treatment Plant

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DECLARATION

I, Ivondia Karumendu, hereby declare that this study is a true reflection of my own research and that this work or part thereof has not been submitted for a degree in any other institution of higher education.

.....Karumendu..... ..03/12/2018.....

Signature Date

CHAPTER 1

1 INTRODUCTION

1.1 Background of the study

In trace chemical analysis, sample pre-treatment techniques are often required to pre-concentrate the target analytes that are present at extremely low concentration levels and eliminate the interference resulting from the complex sample matrix [1]. Polydimethylsiloxane (PDMS) elastomer is the most commonly used polymer in silicone-based extraction methods and is commonly sold as silicone rubber. Sampling devices containing PDMS have been used for the analysis of volatile organic compounds (VOCs) in various matrices [2] and the extraction relies on the sorption of the analytes into the polymeric material [3]. This polymer provides many advantages like the ability to retain apolar organic analytes and to a certain extent, depending on molecular properties and sampling conditions, amongst other, also polar organic analytes. It has a high stability towards temperature, water and a broad range of organic solvents [3]. In addition, only a limited number of well characterised PDMS decomposition products are formed during thermal desorption of the trapped volatiles [2].

The first applications using PDMS as a sorptive material were performed in the 1980s for the extraction of organic substances from water and trapping volatiles in an open-tubular capillary column with cross-linked PDMS [3]. In 1989, solid phase microextraction (SPME) was developed by Arthur and Pawliszyn as a static sorptive sampling technique [3]. SPME is a simple, time-efficient, solvent-free technique that

allows the integration of sampling, isolation and enrichment in one step [4]. The technique uses a short piece of a fused-silica fibre coated with a polymeric stationary phase. The fibre is exposed either to the liquid sample by direct immersion (DI) or to the headspace (HS) of the sample. The analytes are then, either thermally desorbed, or extracted with a small amount of solvent, prior to gas chromatography–mass spectrometry (GC-MS) analysis [3]. Unfortunately, the technique has many shortcomings such as low sensitivity because of the small volume of polymer that is used as sorptive medium, and polar compounds remain a challenge. Other disadvantages include high cost, short lifespan, non-resistance to high temperature and fragility of the fibre. [2].

In order to overcome the low sensitivity of SPME, Baltussen and co-workers introduced stir-bar sorptive extraction (SBSE) in 1999 [5] as an improved technique, where a stir bar is coated with PDMS to extract the analytes [6]. For a decade, PDMS was the only commercially available extraction phase for SBSE [7], however the recovery of polar analytes is poor on PDMS and often derivatization is necessary to increase extraction yields [8], which is one of the main disadvantages of the technique [5]. In recent years, alternative coatings which are more suited to polar analytes have been used in SBSE [6]. These include polyurethane, poly(vinylpyridine/ethylene dimethacrylate, poly(phthalazine ether sulfone ketone), polydimethylsiloxane/ polypyrrole and poly(methyl methacrylate/enthylenglycol dimethacrylate and molecularly imprinted polymer (MIP), which is a tailor-made material with high selectivity for a target compound [6]. Although the operating principles for SPME and SBSE are essentially the same [1], the volume of PDMS coated on the stir bar is 50-250 times larger than that on a SPME fibre, thus high recovery and extraction capacities are obtained with SBSE [9]. However, one of the

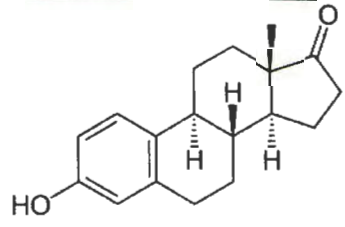
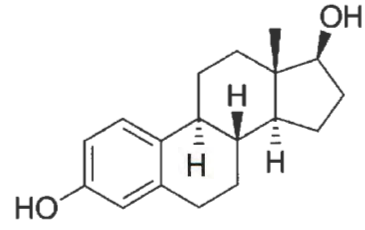
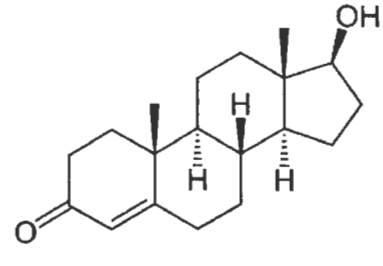
major disadvantages of SBSE is that relatively expensive equipment are required for thermal desorption and cryotrapping to ensure that the desorbed analytes are introduced into the GC column as a narrowly focussed band [2].

In an attempt to overcome the need for high cost automated instrumentation required by SBSE for desorption and cryotrapping, Burger et al [10] developed a sample enrichment probe (SEP). The SEP device is constructed from an inert thin stainless steel stalk/rod with a polydimethylsilicone rubber tubing (sleeve) on one end as the extraction phase. As is the case with SBSE, a fairly large volume of PDMS rubber is used in the SEP technique and in principle, it is capable of generating outcomes comparable to those achieved by SBSE [2]. To analyse the sampled analytes, the SEP is transferred to the heated GC inlet where the compounds are desorbed from the probe. The advantages of the technique are its simplicity and extremely low cost.

Various natural and synthetic chemicals are classified as environmental endocrine disrupting chemicals (EDCs) and the most important being natural and steroidal synthetic hormones and heavy metals [11]. The contamination of steroid hormones in the aquatic environments are mainly through direct discharge and their incomplete removal in wastewater treatment plants (WWTPs) [12, 5, 11]. Estrogens, a subclass of steroids hormones, can be divided into endogenous, natural and artificial or synthetic estrogens and have been associated with reproductive disorder, birth defects, breast cancers and other diseases [13, 14]. Natural estrogenic steroids like estrone (1,3,5(10)-estratriene-17-one) (E1) and 17β-estradiol (1,3,5(10)-estratriene-3,17β-diol) (E2) which are aromatised C_{18} steroids and androgenic steroids like testosterone (4-androsten-3-one) (T) which are C_{19} steroids, are often detected in water samples (Table 1.1) [5, 15]. Estrone and 17β-estradiol can affect the balance of ecological systems even at extremely low concentrations [16] with E2 being the most

potent one [15]. Hormone residues occurrence in wastewater, surface water, groundwater and even drinking water has been increasingly reported [5]. These hormone residues are a cause of concern due to their effects on organisms' normal physiological functions [5].

Table 1-1: Chemical structures of the studied steroid hormones

Steroid	Abbreviation	Structure
Estrone	E1	
17β-Estradiol	E2	
Testosterone	T	

1.2 Statement of the problem

Due to the disruptive effects of the steroid hormones in organisms, it is important for these chemicals to be monitored in water sources, especially in Namibia where wastewater is being reclaimed for drinking purposes. Most existing solventless sampling techniques, based on sorptive extraction using PDMS, have been used for the analysis of hormones and have yielded satisfactory results [5, 8]. However, techniques such as SBSE, where a volume of PDMS material comparable to that of the SEP is employed, make use of expensive desorption and cryotrapping apparatus which needs to be installed on the GC-MS instrument [10]. The SEP is a new, simple, extremely cheap and under-utilised sorptive sampling technique, developed in 2006 and it has been shown that it can yield results comparable to that of SBSE [2, 10]. To the best of our knowledge, there is no previous publication on a SEP method used for the determination of hormones in environmental samples.

1.3 Objectives of the study

The objectives of the study were:

a) To develop a SEP method for the analysis of estrone, 17β-estradiol and testosterone in water using GC-MS.
b) To validate the developed SEP method in terms of precision (reproducibility), linearity, limit of quantification (LOQ) and limit of detection (LOD).
c) To apply the validated method to the quantitative determination of steroid hormones in wastewater and potable water samples.

1.4 Significance of the Study

The SEP method for the analysis of hormones in water developed in this study will offer a low cost, solventless and green analytical alternative to SPME and SBSE. Ultimately the SEP method can be implemented in almost any laboratory that performs water analysis using gas chromatography, and will offer a cost efficient and environmentally friendly alternative to solvent based sample preparation techniques. The simplicity and low cost of the SEP device can be an advantage for developing countries like Namibia.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Sorptive extraction techniques

PDMS (Figure 2-1) was first applied as sorptive phase for the extraction of analytes in the mid-1980s. Later, over the years, methods with similar approaches were developed, including solid phase microextraction (SPME), stir bar sorptive extraction (SBSE), silicone rod (SR) extraction [17] and recently, the sample enrichment probe (SEP) [10] and the fabric phase sorptive extraction (FPSE) [14]. Generally, these approaches decreases sample preparation time and reduce organic solvent consumption, and for that reason, have replaced the solvent-consuming techniques such as SPE [5]. Both these techniques are environmentally friendly and are considered green procedures [17]. SPME and SBSE are widely applied in various analytical fields especially in the environmental analysis of various compounds. Due to these being similar to SEP in extraction mechanism, their applications are discussed further in detail.

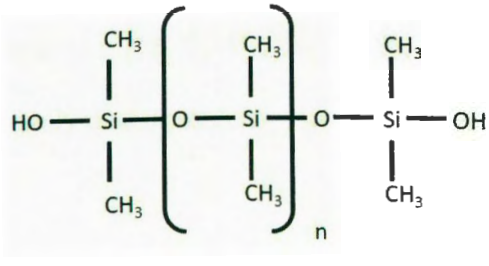


Figure 2-1: Chemical Structure of PDMS

2.1.1 Solid-phase microextraction

Since the introduction of SPME in 1989, it has gain popularity as a sorbent microextraction technique for sample preparation [18]. This miniaturised, solvent-free sample preparation technique is used for the extraction of analytes from gaseous or liquid samples by sorption onto/into a thin polymer coating fixed to the solid surface of a fibre, inside an injection needle or a capillary. SPME is used in either DI or HS mode for extraction of analytes, commonly in combination with GC-MS for the analysis. To achieve all SPME sampling steps (i.e. extraction time, desorption time and fiber cleaning) in one go, the device is coupled to an autosampler, which lead to better precision, repeatability and high throughput [4, 18]. New fiber materials for SPME have been synthesized to improve the quality of the results and applied in different analytical fields [19]. In order to overcome the poor selectivity of the traditional SPME fibers when applied to complex matrix from biological, food and environmental samples, molecularly imprinted polymers (MIP) sorbents are used as fiber coatings. These sorption materials have high selectivity for certain group of analytes and affinity for one type of structure. The MIPs properties are thermal and pH range (0-14) stability, which is due the design factors including the choice of monomer, cross-linker and polymerisation method. MIPs are achieved by the interaction between the monomer molecule functional groups and a template molecule [18, 5, 19, 20]. Some of the SPME applications in different areas are briefly discussed in the following sections.

2.1.1.1 SPME applications in environmental analysis

Direct immersion solid phase microextraction (DI-SPME) technique was used to determine 16 pesticides (six organophosphorus – trichlorfon, diazinon, methyl parathion, fenthion and ethyon; three pyrethroids – bifenhin, permethrin, cypermethrin; two imidazoles – imazalil and prochloraz; two strobilurins – azoxystrobin and pyraclostrobin; one carbamate – carbofuran; one tetrazine – clofentezine, and one triazole – difenoconazole) in groundwater samples [21] and steroids (mesterone, methandriol, estrone, estradiol, androstendione, prasterone and diethylstilbestrol) in water at parts-per-trillion and lower concentrations [15]. For the simultaneous determination of residues of the pesticides, the method showed good selectivity and precision with linearity between 0.05 and 250 ng/mL. Ultra-trace detection levels ranging from 30–200 pg/L with good linearity from 0.01–5 ng/ml were demonstrated by steroids analysis on DI-SPME-GC-MS-MS.

In another study a simple online headspace solid-phase microextraction (HS-SPME) method coupled with gas chromatography-mass spectrometry for the simultaneous determination of trace amounts of nine estrogenic odourant alkylphenols and chlorophenols and their derivatives in water samples was developed [22]. The extraction conditions, including fibre selection, affecting the extraction efficiency of the HS-SPME method were optimised. Of all the fibres evaluated, the Divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre was the one that showed the best results. The optimised method showed good linearity ($R^2 >$ 0.989) and good recoveries (72-126%) with very low limits of detection (0.002-0.5 µg/L).

Headspace and direct immersion solid-phase microextraction was applied in the extraction of organothiophosphates related to the Chemical Weapons Convention (CWC) from water and complex matrices [23]. Analyses were performed on a gas chromatographic system coupled to a tandem mass spectrometer (GC-MS/MS). DI-SPME-GC-MS/MS approach was shown to be the suitable technique for the detection of different organophosphates related to the CWC from complex matrices.

An on-fiber silylation (derivatisation) SPME-GC-MS method was applied for the determination of exogenous endocrine disrupting chemicals (octylphenol – OP, nonylphenol – *t*-NP, diethylstilbestrol – DES) and endogenous steroid hormones (dehydroisoandrosterone – DEHA, estrone – E1, 17β-estradiol – E2, testosterone – T, pregnenolone – PREG) in environmental aqueous (river water) and biological samples (fish serum) [24]. The optimised method showed good linearity ($R^2 > 0.996$) and limit of detection (0.001-0.118 μg/L).

In-tube SPME is an automated SPME technique which uses an open tubular fused-silica with an inner surface coating as a sampling device. This technique requires lower sample volumes and has better precision and sensitivity than conventional SPME [5, 25]. Anabolic steroids (boldenone, nandrolone, testosterone, methyltestosterone, epiandrosterone, androsterone, stanozolol) from human urine samples were analysed using an on-line *in-tube* solid phase microextraction liquid chromatography-mass spectrometry (SPME-LC-MS) extraction method [25]. The method provided good linearity (correlation coefficient over 0.995), however the limits of detection (LODs) were found to be in high range of 0.009 to 0.182 ng/mL. Using the same SPME technique for the simultaneous determination of four endocrine disruptors (17β-estradiol, estriol, bisphenol A and 17α-ethinylestradiol) in environmental water, Wen and co-workers coupled it with high performance liquid

chromatography with fluorescence detection (HPLC-FLD) [26]. As the extraction medium for the SPME fibre, a poly(acrylamide-vinylpyridine-N,N'-methylene bisacrylamide) monolith was chosen. The limits of detection and quantification were found to be in the low range of 0.006 – 0.10 μg/ml and 0.02 – 0.35 μg/ml, respectively.

Five fluoroquinolones (FQs) in cultured puffer fish were determined using a simple and rapid method based on *in vivo* solid-phase microextraction coupled with liquid chromatography-tandem mass spectrometry (SPME-LC-MS/MS) [27]. The analytes were extracted using a custom-made biocompatible C18-PAN fibre as sorbent material. The graphs were linear in the range of 2-1000 ng/g, with $R^2 > 0.998$ and detection limits ranging from 0.3 to 1.5 ng/g (S/N = 10) for all FQs.

An automated headspace solid phase microextraction comprehensive two dimensional gas chromatography with time of flight mass spectrometry (HS-SPME-GC×GC-ToF/MS) method was applied in the determination of steroids, caffeine and methylparaben in water [28]. Two fiber coatings (polydimethylsiloxane-PDMS and polydimethylsiloxane/divinylbenzene-PDMS/DVB) were tested and the preferred fiber was PDMS/DVB, which is a mixed polymeric phase as it successfully extracted all the studied analytes. Under optimum conditions, the calibration graphs were linear in the range of 1.2-120 μg/L and 2.1-50 μg/L for E1 and E2, respectively. The limits of detection were 0.02 and 1.34 μg/L for the two compounds.

2.1.1.2 SPME applications in food analysis

A simple and rapid DI-SPME-GC-MS method for the analysis of some endocrine disrupting chemicals (4-tert-octylphenol, 4-octylphenol, 4-nonylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, bisphenol A) leaching from baby milk feeding bottles was developed [29]. Under optimal conditions, the limit of detection ranged from 0.02 to 0.12 µg/mL. High recoveries of 85-102 % were obtained and the method showed satisfactory precision (%RSD, <5.9% for intra-day and <7.9% for inter-day precisions). Using the same method free trans-resveratrol in commercial wines, spirits and grapes was analysed by liquid chromatography with ultra violet diode-array detection (LC-UV) [30]. The concentration levels were found to range from 0.007 to 4.486 µg/mL in all the analysed samples. Limits of detection and quantification in real samples varied from 0.5 to 1.1 ng/mL and 1.6 to 3.7 ng/mL, respectively. They proposed that the method could be easily transferred in the production facility and would be useful for many purposes.

Headspace solid phase microextraction-chiral gas chromatography-mass spectrometry (HS-SPME-GC-MS) method using the CAR/DVB/PDMS fibre was employed to investigate 12 pairs of familiar volatile terpenoid enantiomers in different teas [31]. Good LODs in the range of 0.11-1.59 ng/L were obtained with correlation coefficient (R^2) >0.993. Free and glycosidically bound volatile compounds in sun-dried raisins made from grapes with different fragrance intensities were analysed using the same method [32]. Three SPME fibers (DVB/CAR/PDMS, PDMS/DVB and CAR/PDMS) were investigated and DVB/CAR/PDMS showed the

best results. The obtained LODs were calculated based upon the signal-to-noise ratio and ranged between 0.001 and 47.9 µg/L for the monitored compounds.

2.1.1.3 SPME applications in medical and pharmaceutical analysis

A headspace SPME method was developed by González and co-workers [33] and consequently successfully applied for the simultaneous determination of BTEX (benzene, toluene, ethylbenzene, *p*-xylene) and their metabolites in teeth as environmental biomarkers. Analysis was carried out by HPLC (aromatic acids) or GC-MS (BTEX and phenols), to avoid derivatization and minimize sample preparation time. Satisfactory linearity ($R^2 > 0.984$) was achieved with limits of detection ranging from 0.2-33.3 ng/mL and 0.06-0.09 pg/mL for SPME-HPLC and SPME-GC-MS, respectively. These results showed that the SPME method was suitable for the analyses of trace BTEX and phenols in human teeth.

2.1.1.4 Modified versions of SPME and new fibre materials

To overcome the problems related to the SPME fibre fragility [5] and to improve the quality of the results [19], a series of new versions of the technique have been developed [5]. Metal ions combined with organic linkers crystalline solids called metal-organic frameworks (MOFs) have emerged as new molecular sieve materials with high porous framework and have been used as SPME fiber coatings. MIL-53(M), where M can be Fe, Al or Cr, is a MOF, material of Institute Lavoisier (MIL)

reputed to have a flexible structure which optimises interaction between guest molecules and the framework [34]. NH_2-MIL-53(Al) was synthesised and used as an SPME fiber coating, subsequently successfully applied for the determination of five synthetic musks (SMs) and eight organo-chlorine pesticides (OCPs) in environmental water samples by GC-MS [34]. The SPME fibre was used in the direct immersion extraction mode. The NH_2-MIL-53(AL)-coated fibre produced low LODs (0.025-0.83 ng/L for SMs and 0.051-0.97 ng/L for OCPs) and good linearity under optimised conditions.

Highly selective antibodies prepared on solid support such as fused silica fiber and capillary, known as the immunoaffinity SPME (IA-SPME) is a form of immunoaffinity chromatography (IAC) [35]. Immunoaffinity solid phase microextraction rods for the extraction of diethylstilbestrol (DES), hexostrol (HES) and dienestrol (DIS) in environmental water were developed [35]. Stainless steel rods were coated with porous silica particles, and then anti-diethylstilbestrol monoclonal antibody (mAb) was employed onto the rods using the antibody immobilization approach. Samples were analysed by ultrahigh-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Under the optimised conditions, the IA-SPME-UPLC-MS/MS showed good linearity (correlation coefficients over 0.996) with LODs ranging from 0.05-0.15 ng/mL for the three estrogens.

An innovative PAL (Prep and load solution) solid phase microextraction (PAL-SPME) device has been developed by Kremser et al [36], and then applied for the extraction of freely dissolved polycyclic aromatic hydrocarbons (PAHs) in water. The PAL sampler is composed of a stainless steel backbone with an arrow-shaped tip and enlarged sorption phase (30 mm × 250 µm) compared to the 10 mm × 100 µm

sorption phase of a classical SPME fiber. The analytes were determined by GC-MS. Under optimal conditions, PAL-SPME MDL (method detection limits) ranged from 0.1-0.8 ng/L and the RSD were found to be in the range of 5.5-11.9 % at 10 ng/L for target analytes. Results of the PAL-SPME were compared to classical SPME fibers and SBSE bars data, and are reported to be similar to SBSE and better to those of classical SPME.

Conventional SPME is coupled to performance reference compounds (PRCs) for measuring freely dissolved concentration (C_{free}) for the risk assessment of hydrophobic organic contaminants (HOCs) [37]. Disposable solid phase microextraction PDMS fibers (35µm and 100µm coating) was coupled with performance reference compounds (PRC-SPME) for the analysis of freely dissolved dichlorodiphenyltrichloroethane (DDT) and metabolites in seawater by Lin et al [37]. The disposable SPME fibers were preloaded with stable isotope for *in situ* sampling. The *in situ* PRC-SPME method showed practical results and the feasibility of the method.

A nanostructural-solid phase microextraction coated fibre was applied to the extraction of benzene, toluene, ethylbenzene and xylene (BTEX) from water samples for gas chromatography-mass spectrometry analysis [38]. PDMS sol-gel solution was converted to nanoparticles on a stainless steel wire by the electrospinning technique. SPME was used in headspace mode for the extraction. The linear range and limit of detection for all compounds were found to be 1-5000 µg/L and 0.3-5 µg/L, respectively.

A novel method called SPME Arrow system was coupled with GC-MS and introduced for the analysis of volatile low molecular weight alkylamines

(dimethylamine hydrochloride and trimethylamine hydrochloride) in ambient air and wastewater [39]. The SPME arrow system uses a larger volume of the sorbent compared to a standard SPME fiber. The limit of quantification for dimethylamine hydrochloride and trimethylamine hydrochloride were 10 µg/L and 0.13 µg/L while the reproducibility expressed as relative standard deviation was 12% RSD and 14% RSD, respectively.

In the application of the MIP technique, a testosterone-imprinted polymer coated SPME fiber was used for the extraction of anabolic steroids (androsterone, stanolone, androstenedione and methytesosterone) in spiked human urine [20]. Testosterone, methacrylic acid (MAA) and trimethylol-propanetrimethacrylate (TRIM) were used as a template molecule, functional monomer and cross-linker, respectively. The selective molecularly imprinted polymer coated solid phase microextraction (MIP-SPME) was coupled with GC-MS for analysis. The limit of detection was in the range of 0.2–0.8 µg/L, and the recoveries were between 80.1 and 108.4 %. Hu et al [40] used multiple co-polymerisation method to produce a 17β-estradiol coated SPME fibre, which was subsequently used for the extraction of estrogenic compounds (estriol, estrone, 17-β-estradiol and 17β-ethynylestradiol) in fish and shrimp tissue samples. The MIP-coated SPME method was coupled directly with HPLC for analysis. Under optimum conditions, the obtained limits of detection were in the range of 0.98 – 2.39 µg/L, whereas the recoveries were 80.0 – 83.64 % and 85.0 – 94.1 % for fish and shrimp tissues samples, respectively. The calibration graphs were linear in the range of 5.0-30 µg/L (estriol and 17-β-estradiol) and 15.0-40 µg/L (estrone and 17β-ethynylestradiol).

2.2.2 Stir-bar sorptive extraction

Stir bar sorptive extraction (SBSE), like SPME, is also an easy technique which allows effective pre-concentration and extraction of the analytes from sample matrices [18]. In this technique, the extraction phase is placed on a magnetic bar which is also known as the Twister® [5]. SBSE can be used in both the direct immersion and headspace modes. The analytes accumulated on the coated stir bar can be recovered by thermal desorption (TD) followed by GC (this enhances sensitivity) [7] or liquid desorption (LD) is used as an alternative [8]. Although SBSE was originally intended for environmental analysis it has over time been applied to almost every analytical chemistry field [41]. Some of the applications in the different fields are briefly discussed in the following sections.

2.2.2.1 SBSE applications in environmental analysis

In environmental analysis, SBSE has been mostly used in water and soil matrices for the determination of persistent organic pollutants (POPs) [41]. A sensitive and simple method based on SBSE coupled with liquid chromatography tandem mass spectrometry (SBSE-LC-MS/MS) for the determination of tributyltin in seawater at ultra-trace levels was developed [42]. The optimised method showed good recoveries ranging from 92-102 %. The obtained limit of detection and limit of quantification were 0.8 and 2.5 ng/L, respectively. Later, the same authors [43] employed a stir bar sorptive extraction and thermal desorption with gas chromatography-triple quadrupole mass spectrometry (SBSE-TD-GC-MS/MS (QqQ)) method to determine

77 priority persistent organic pollutants in river water. Under optimum conditions, the LOQs were between 0.14 and 10 ng/L with recoveries of up to 111%. SBSE-LC-MS/MS was also used in the determination of 15 pesticides and metabolites in surface water [44]. The validated method limit of quantification ranged from 0.02 to 1 μg/L for the target compounds.

The extraction efficiency of stir bar sorptive extraction was compared to two other pre-concentration techniques [dispersive liquid-liquid microextraction (DLLME) and solid sphase extraction (SPE)] in the analysis of six hypolipidaemic statin drugs (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin) in wastewater and river water by high performance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (HPLC/Q-TOF-MS) [45]. The three procedures results were similar for the concentration of statin drugs with the method detection limit in real sample in the range of 0.52-2.00 ng/L, 0.04-11.2 ng/L, 0.10-17.0 ng/L for SBSE, SPE and DLLME, respectively.

The extraction efficiency of direct immersion stir bar sorptive extraction (DI-SBSE) and headspace stir bar sorptive extraction (HS-SBSE) methods followed by automated thermal desorption (TD) coupled to gas chromatography-mass spectrometry (GC-MS) were studied for the determination of eighteen (18) synthetic musk fragrances in natural waters (surface and ground) and wastewater [46]. To develop a sensitive and specific method for quantification of 18 synthetic fragrances listed in a homebuilt retention time locking (RTL) musk database in water samples, a combination of elements of SBSE-TD/RTL-GC-MS was applied. Operating the MS-detector in full scan mode, the DI-SBSE-TD/RTL-GC-MS method produced good linearity and repeatability (with the %RSD range of 3.7-23.5 %) for all musks, with detection limits at the ng/L levels.

A novel off/on-site stir bar sorptive extraction approach with a home-made portable electric stirrer was developed by Mao et al [1] for the analysis of polycyclic aromatic hydrocarbon compounds in environmental water by high performance liquid chromatography-fluorescence detection (HPLC-FLD). The portable SBSE sampling device mainly consisted of a miniature electric stirrer with a stirring speed regulator and a magnetic stir rod welded below. The SBSE was used in the direct immersion (DI), headspace (HS) and continuous flow (CF) modes. The method was reported as the most sensitive compared to literatures for PAHs determination. All three extraction modes produced good linearity with LODs in the range of 0.05-3.41 ng/L, 0.03-2.23 ng/L and 0.09-3.75 ng/L for DI-SBSE, HS-SBSE and CF-SBSE, respectively.

Another novel hyphenated technique method of isotope dilution SBSE combined with direct analysis in real time Orbitrap^{TM} mass spectrometry (DART/OT-MS) for the analysis of phosphoric acid esters from aqueous samples was reported by Bridoux and co-workers [47]. To accomplish the connection of SBSE to DART/Orbitrap-MS, the twister was placed in the middle of an open-ended glass tube between the DART plasma outlet and the entrance of the LTQ-Orbitrap mass spectrometer. The SBSE/DART/Orbitrap-MS method showed good linearity in the concentration range studied (0.1-750 ng/mL).

2.2.2.2 SBSE applications in food analysis

The application of SBSE in food analysis has been mostly to the analysis of pollutants and toxins with little undertakings in nutrients analysis [41]. A stir bar

sorptive extraction with thermal desorption-gas chromatography-mass spectrometry (SBSE-TD-GC-MS) method was applied for the determination of bisphenol A (BPA), bisphenol F (BPF), bisphenol Z (BPZ) and biphenol (BP) in canned beverages and filling liquids of canned vegetables [48]. Derivatisation of the SBSE bar was carried out by both *in situ* acetylation and *in tube* silylation. The optimised method provided detection limits between 4.7 and 12.5 ng/L and the intraday and interday precisions lower than 6%. A stir bar sorptive extraction with thermal desorption-retention time locked-capillary gas chromatography-mass spectrometry (SBSE-TD-RTL-capillary GC-MS) method was used in the multi-residue screening of pesticides in vegetables, fruits and baby food [49]. More than 300 pesticides in the three different matrices could be monitored with the SBSE-TD-RTL-capillary GC-MS operated in the scan mode. The method produced detection limit in the range of mg/kg (ppm) to the sub-µg/kg (ppb) levels.

SBSE-LC-DAD (stir bar sorptive extraction before liquid chromatography and diode array detection) was used for the analysis of strobilurin (metominostrobin, azoxystrobin, dimoxystrobin, kresoxim-methyl, picoxystrobin, pyraclostrobin, trifloxystrobi) fungicides in fruit (apple, pear, grape, lemon) samples [50]. The optimised method showed good repeatability (%RSD below 11%), recoveries (of 80-105 %) and detection limits ranging between 5 and 10 ng/mL.

A multi-stir bar sorptive extraction (^{m}SBSE) coupled by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) method for the determination of odour compounds in aqueous samples was developed [7]. In this ^{m}SBSE concept, two twisters [an ethyleneglycol-modified silicone (EG Silicone) and PDMS] were used to simultaneously extract polar and non-polar compounds, respectively. During extraction the EG Silicone stir bar is attached on the inner side wall of the vial while

the more robust PDMS stir is stirring at the bottom in order to reduce friction and preserve the soft coating of the EG Silicone stir bar. The stir bars were then placed in the same glass liner for desorption after extraction. The achieved limit of detection for the '''SBSE- TD-GC-MS method ranged from 0.011 to 0.071 ng/mL. The same concept of using two stir bars in a single sample solution was applied for the simultaneous determination of preservatives with different polarities in beverage samples by high performance liquid chromatography [51]. The dual-phase dual stir bar used were coated with 3-aminopropyltriethoxysilane-hydroxy-terminated silicone oil (APTES-OH-TSO) and C_{18}-PDMS, synthesised by sol-gel technique and adhesion, respectively. The six compounds studied were benzoic acid (BA), sorbic acid (SA), methyl *p*-hydroxybenzoate (MP), ethyl *p*-hydroxybenzoate (EP), propyl *p*-hydroxybenzoate (PP) and butyl *p*-hydroxybenzoate (BP). The LODs for the target analytes were found ranging from 0.6-2.7 µg/L and the RSDs for ($C_{BA,SA}$ = 5 µg/L, C_{MP} = 20 µg/L, $C_{EP,PP,BP}$ = 10 µg/L) ranged from 4.6 to 9.2 % (n = 7).

2.2.2.3 SBSE applications in medical and pharmaceutical analysis

Due to the very complex nature of sample matrices, limited sample volumes (which are often in a few mL/µL), and lack of suitable sorbent materials (substances are mostly polar compounds), SBSE has been least applied in the field of medical and pharmaceutical analysis [41]. A HS-SBSE-GC/MS method was developed and then applied for the chemical characterisation the vapour phase components (volatiles and semi-volatiles) of diluted cigarette smoke generated by the Borgwaldt RM20S smoking machine used in combination with the BAT exposure chamber [52].

Thermal desorption was used to recover the adsorbed analytes. The method allowed identification of sixteen compounds and was reported as simple and cost effective which can be employed to a wide range of analyses.

2.2.2.4 SBSE applications with new coating materials

To improve the application of SBSE, new coatings which allow sorption of polar compounds have been developed [41]. Methods generally used in the fabrication of the new coatings include sol-gel techniques, and molecularly imprinted technology (MIT). Examples of the new materials used as sorbents are graphene and derived materials, restricted access materials (RAMs), immunosorbents, molecularly monoliths (MIMs), molecularly imprinted polymers (MIPs) [53].

A polyaniline/hydroxyl multi-walled carbon nanotube (PANi/MWCNTs-OH) composite-coated stir bar was prepared and coupled with HPLC-UV for the simultaneous determination of polar and apolar compounds (phenols, non-steroidal anti-inflammatory drugs and polychlorinated biphenyls) in environmental samples [54]. The results indicated that the method was suitable for both non-polar and polar analytes in various sample matrices. The achieved LODs were in the range of 0.09-0.81 μg/L.

Due to their interesting structures, numerous types of metal-organic frameworks (MOFs) such as MOF-99, MOF-5(Zn), ZIF-8, MIL-53(Al) and MAF-X8 have been synthesized, and applied as sorbents for diverse analytes in various fields [55]. A composite magnetic material, Fe_3O_4-MOF-5(Fe), was synthesised using the solvothermal method and then employed as a coating through magnetic adhesion

approach for the Nd-Fe-B permanent magnet stir bar [55]. The MOF coated stir bar was used for the extraction of six polychlorinated biphenyls (2,2',5,5'-tetrachlorobiphenyl, 2,2',4,5,5'-pentachlorobiphenyl, 2,2',3,4,4'5,-hexachlorobiphenyl, 2,3,4,4,5-pentachlorobiphenyl, 2,2',4,4'5,5'-hexachlorobiphenyl, 2,2',3,4,4'5,5'-heptachlorobiphenyl) in fish samples. Analyses of the compounds were carried out on the gas chromatography-mass spectrometry system. The calibration graphs were linear in the range of 0.01-500 µg/L and detection limits between 0.061-0.095 ng/g. MOF-SBSE-GC-MS method produced satisfactory recoveries (94.3-97.5 %) for all the PCBs.

Using the sol-gel technique, three novel polydimethylsiloxane/metal-organic framework termed PDMS/MOFs (PDMS/MOF-5, PDMS/MOF-199 and PDMS/IRMOF-3) types of coatings were prepared and their extraction efficiency was evaluated for the extraction of seven estrogens (17-β-Estradiol, dienestrol, diethylstilbestrol, Estrone, 4-t-octylphenol, bisphenol-A, 17-α-Ethynylestradiol) in environmental water samples [56]. Under optimum conditions, the SBSE (PDMS/IRMOF-3)-HPLC-UV method showed good linearity in the range of 2-2500 µg/L for 17-α-Ethynylestradiol and 1-2500 µg/L for all other estrogens, with LODs ranging between 0.15 and 0.35 µg/L. The same authors developed a new method employing a polydimethylsiloxane/polythiophene (PDMS/PTH) coated stir bar for the extraction of organophosphorus pesticides (OPPs) in environmental water samples [57]. SBSE extraction efficiency of the OPP's (phorate, fenitrothion, malathion, parathion, quinalphos) was assessed by liquid desorption-large volume injection-gas chromatography-flame photometric detection (LD-LVI-GC-FPD). The limit of detection was 0.011-0.038 µg/L (all OPPs), RSD was 4.0-9.8 % (n = 8, c = 1 µgL) and linearity was 0.2-100 µg/L (phorate) and 0.1-100 µg/L (other 4 OPPs).

Another type of modified coated stir bar, a poly(dimethylsiloxane)/β-cyclodextrin/divinylbenzene (PDMS/β-CD/DVB) was used by the same authors for the analysis of four estrogens from animal-derived food by liquid desorption (LD) and high performance liquid chromatography with ultraviolet (HPLC-UV) detection [58]. The PDMS/β-CD/DVB-SBSE-LD-HPLC-UV method under the optimum conditions showed excellent linearity (2 – 2000 μg/L), limits of detection (0.21 – 1.6 μg/L) and the RSD for 10 μg/L ranged between 8.4 and 11.7 % (n = 8). The developed method was successfully applied to the analysis of estrogens in pork and chicken sample.

Other stir bar coating, also prepared by sol-gel technology by introducing different groups in the PDMS were applied in various studies. A pH-resistant organic-inorganic hybrid titania-hydroxy-terminated silicone oil (titania-OH-TSO) coated stir bar extraction efficiency was compared to polydimethylsiloxane (PDMS), polydimethylsiloxane-divinylbenzene (PDMS-DVB), polydimethylsiloxane-β-cyclodextrin (PDMS-β-CD) and C18 coated stir bar for the extraction of drugs of abuse (amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, ketamine) in urine samples [59]. Analysis was performed with a high performance liquid chromatography-ultraviolet visible detection. For the extraction of amphetamines and ketamine, the titania-OH-TSO coated stir bar showed better results with LODs in the range of 2.3-9.1 μg/L. A Ni:ZnS-AC with 1-ethyl-3-methylimidazolium hexafluorophosphate ionic liquid (IL) modified stir bar was applied for extraction and pre-concentration of losartan (LOS) and valsartan (VAL) from biological matrices before their analysis with HPLC [60]. The optimised method provided linear ranges of 0.4-50 μg/L and 0.5-50 μg/L, and limits of detection of 0.12 and 0.15 μg/L for losartan and valsartan, respectively. The

RSDs for 5 μg/L of LOS and VAL were 4.4 and 4.9% (n = 6). A combination of SBSE-HPLC-UV/ICP-MS was used for the analysis of amphoteric thyroxine (3,3',5,5'-tetraiodothyronin, 3,3',5-triiodothyronine, reversed-3,3',5-triiodothyronine) and their metabolite (3,5-diiodothyronine) in urine samples [61]. Polyethyleneglycol/hydroxyl PDMS/γ-mercaptopropyl trimethoxysilane (PEG/OH-PDMS/γ-MPTS) was employed as a coating for the stir bar and the coated stir bar showed good durability (could be re-used for more than 20 times) without the loss of adsorbed analytes. The LODs for the target compounds ranged between 0.60-2.20 μg/L and 0.0071-0.0355 μg/L for the SBSE-HPLC-UV and SBSE-HPLC-ICP-MS methods, respectively. A simple and rapid method for the analysis of steroid hormones (estriol, estradiol, ethynylestradiol, estrone, progesterone, medroxyprogesterone, levonorgestrel, northindrone) in water matrices was developed by using a PDMS/phenyltrimethylsiloxane/β-cyclodextrin coated stir bar coupled to a laser diode thermal desorption/atmospheric chemical ionisation tandem mass spectrometry (LDTD-APCI-MS/MS) [9]. In all the water matrices studied (HPLC grade water, tap water and raw wastewater), the recoveries of all the compounds ranged from 55 – 96 %, except estriol which showed poor recovery values (< 2%). The method detection limits were within the range of 0.1 – 0.3 μg/L, except for estriol which was at 48 μg/L. The extraction performance of the stir bars was unchanged for at least over 50 extractions.

Eleven quinolones in bovine milk were determined using immunoaffinity stir bar sorptive microextraction coupled with liquid chromatography-fluorescence method [62]. The immunoaffinity stir bar was constructed by immobilizing mAbs to glass bars through covalent linking using glutaraldehyde (GA) as a coupling reagent. The detection limits for individual quinolones ranged between 0.05-0.1 ng/g. The method

inter-day and intra-day precisions ranged from 5.2-12.5 % and 3.2-11.9 %, respectively, and it was confirmed that it can be applied in food safety analysis. The method is environmental friendly as less than 900µL of organic solvent was used in sample preparation.

Monolithic materials have high permeability due to polymerisation of a monomer mixture with a porogen solvent during their preparation [8]. An *in-situ* copolymerisation of methacrylic acid stearyl ester and ethylene dimethacrylate in the precense of a porogen solvent containing 1-propanol and 1,4-butanediol was used to produce a monolithic material for SBSEM-HPLC-DAD method for the determination of six steroid sex hormones in urine matrix at trace level [63]. In comparison to the existing extraction methods for steroid sex hormones, the method was said to be simple, rapid and precise. The limit of detection and limit of quantification were found to be in the range of 0.062 – 0.38 µg/L and 0.20 – 1.20 µg/L, respectively. A different anionic exchange (AE) monolith, prepared by *in situ* copolymerisation of 2-(methacryloyloxy)ethyltrimethylammonium chloride and divinylbenzene was used by the same authors as a sorbent material for stir bar sorptive extraction [64]. An SBSE-AE coupled with liquid desorption ion chromatography with conductivity detector (SBSE-AE/LD-IC/CD) method was developed, subsequently applied to the trace analysis of anions (Br^-, NO_3^-, PO_4^{3-}, SO_4^{2-}) in commercial purified water. Under the optimum conditions, the method showed good linearity ($R^2 > 0.98$) with detection limits within the range of 0.92-2.62 µg/L.

Molecularly imprinted polymer (MIP) coated stir bar have been applied in various studies. A novel method for the molecularly imprinted polymer (MIP)-coated stir bar sorptive extraction of β_2-agonists (ractopamine, isoxsuprine and clenbuterol) in

complex (pork, liver and feed) samples was developed [65]. Samples were analysed by high performance liquid chromatography. The MIP coated stir bar durability was at least 40 times without apparent damage. Linear ranges were 0.5-40 μg/L (ractopamine) and 1.0-40 μg/L (isoxsuprine and clenbuterol), with detection limits ranging from 0.10 to 0.21μg/L. A water-compatible graphene oxide (GO)/molecularly imprinted polymer (MIP) coated stir bar was prepared by *in situ* polymerisation and combined with high performance liquid chromatography-ultraviolet detector for the analysis of propranolol in urine samples [66]. Under optimised conditions the limit of detection was about 0.37μg/L.

2.2.3 Sample enrichment probe applications

The SEP technique is similar to SPME and SBSE in extraction mechanism and uses fairly large volumes of PDMS as is the case with SBSE. To accommodate the insertion and removal of the SEP device, during thermal desorption of the analytes from the sorptive phase and after desorption the GC injector is opened to the atmosphere [10]. The SEP device has not been applied in many published studies yet.

A new sampling technique termed high-capacity sample enrichment probe (SEP) was coupled with gas chromatography for the enrichment and analysis of volatile and semi-volatile organic compounds (n-alkanes) in gaseous and aqueous samples [10]. A SEP device was constructed by fitting a laboratory-grade PDMS rubber tubing (1.00 mm I.D. × 1.75 mm O.D.) on a stainless-steel rod and used as a sorbent. Using two different GC columns FSOT B and glass open-tubular C, the relative standard

deviations (n = 5) for 1 µg/L of the target compounds were in the range of 1.46-7.49 % and 0.49-9.60 %, respectively. The same authors applied an improved SEP device for the extraction of volatile organic compounds from gaseous and aqueous samples [2]. Polyimide-coated fused silica stalks instead of stainless steel stalks and thinner PDMS tubing (0.64 mm I.D. × 1.19 mm O.D.) were used. Analysis of the compounds was performed by GC and GC-MS. Acceptable RSDs were achieved for all the target analytes. The analytes desorbed at a faster rate from thinner sleeve, which reduces the risk of carry-over with the new SEPs.

2.2.4 Other Sorptive Extraction Methods

2.2.4.1 Fabric Phase Sorptive Extraction

A modern sample preparation technique was recently developed by Kabir and Furton, known as fabric phase sorptive extraction (FPSE) [67] to overcome the low sample capacity and longer sample preparation time limitations of the current sol-gel SPME formats [12], and as a response to the global call for making green sample preparation technologies, as FPSE is considered as a microextraction technique [68]. The technique uses the advantages of sol-gel technology derived microextraction sorbents and rich surface chemistry of cellulose cotton fabric substrates to produce an extraction media with abundant advantages [12, 14, 69, 70, 71, 72]. To allow the incorporation of large amounts of sorbent inside the cellulose/polyester substrate, FPSE consists of a flexible and permeable media (2.5 × 2.0 cm) coated with a sol-gel hybrid inorganic-organic sorbent, and this gives it a large surface area for extraction

[68, 72, 73]. Sorption and desorption of the analytes is made better by the open geometry of the FPSE and these are performed by immersing the media into the sample and elution with a suitable solvent, respectively [67]. The advantages of FPSE include: simple, fast, use of minimal solvent, can be submerged directly into raw sample matrix (even with biomasses) without sample pre-treatment requirement, possess a high sample capacity, high pH stability (pH 1 – 12) and can efficiently extract both polar and non-polar analytes [14, 71, 72, 68]. This new sample preparation technique is claimed to be the solution to the major drawbacks of SPME and SBSE techniques [70, 72]. Natural fabric is used as a substrate in FPSE compared to the fused silica or metal wire used in conventional SPME. As in SPME, FPSE can be used in equilibrium, but it can also be used in exhaustive mode similar to SPE [67]. It has an extraction capacity of approximately 400 and 10 times higher than SPME and SBSE, respectively [69, 70]. Even though it's a fairly a new technique, it is already being employed in the extraction of a variety of analytes in different fields of study, including environmental [73, 74], food [67] and medical & pharmaceutical [68] analysis. Some of the applications are briefly discussed below.

A group of pharmaceuticals and personal care products (PPCPs) from environmental water (river water, effluent and influent wastewater) samples were extracted using dynamic fabric phase sorptive extraction followed by liquid chromatography-tandem mass spectrometry (FPSE-UHPLC-MS/MS) [73]. For most compounds the recoveries were more than 60% except the most polar ones whose recoveries ranged between 8 and 38 %. The same method was applied for the determination of six benzotriazole UV stabilizers (BUVSs) in seawater samples [72]. Under optimum conditions the method limits of detection and limits of quantification were in the range 1.06 – 8.96 ng/L and 3.54 – 29.9 ng/L, respectively. Again FPSE-UHPLC-

MS/MS was applied for the determination of four progestogens (norethisterone, norgestrel, megestrol acetate, progesterone) and six androgens (boldenone, nandrolone, androstenedione, dehydroepiandrosterone, testosterone, androsterone) in environmental and biological samples [12]. Satisfactory limits of detection between 1.7 and 264 ng/L were obtained. Santana-Viera et al [68] used the same method (FPSE-UHPLC-MS/MS) for the analysis of seven cytostatic drug compounds (methotrexate, gemcitabine, etoposide, cyclophosphamide, vincristine, vinblastine and tamoxifen) commonly used in anti-cancer therapies. The method LOD and LOQ ranged from 0.20 – 80 ng/L and 0.68 – 267 ng/L, respectively. The method showed higher than 40% recoveries and good linearity (RSD less than 12%).

Three brominated flame retardants (BFR's) [tetrabromobisphenol A, tetrabromobisphenol A bisallylether, tetrabromobisphenol A bis(2,3-dibromopropyl)ether] were analysed by Huang et al [70] using two extraction techniques including stir bar fabric phase sorptive extraction coupled with high performance liquid chromatography (stir bar-FPSE-HPLC) and magnetic stir fabric phase sorptive extraction coupled with high performance liquid chromatography (magnetic stir-FPSE-HPLC). The cellulose fabric surface was prepared with poly (tetrahydrofuran), poly (ethylene glycol) and poly (dimethyldiphenylsiloxane). Both techniques yielded high extraction capability with low limits of detection (0.01 – 0.05 μg/L) and high recoveries (90 – 99 %).

A fast and sensitive method was developed for the quantification of biologically important molecules (17α-ethynyletradiol, β-estradiol and bisphenol A) using fabric phase sorptive extraction coupled to high performance liquid chromatography with fluorescence detection (HPLC-FLD) [14]. The method showed good linearity (R^2 > 0.992) with detection limits within the range of 20 – 42 pg/mL. In the quantification

of some endocrine-disruptor alkyl phenols (4-*tert*-butylphenol, 4-s*ec*-butylphenol, 4-*tert*-amylphenol and 4-cumylphenol) in aqueous and soil samples the same authors [74] developed a novel method using fabric phase sorptive extraction with high-performance liquid chromatography with ultraviolet detection (HPLC-UV). The detection limits ranged from 0.161 – 0.192 ng/mL with good coefficient values (R^2 > 0.992) and recoveries were 74.0, 75.6, 78.0 and 78.3 % for 4-*tert*-butylphenol, 4-s*ec*-butylphenol, 4-*tert*-amylphenol and 4-cumylphenol, respectively.

An ionic liquid immobilized fabric phase sorptive extraction method coupled with high performance liquid chromatography for rapid screening and simultaneous determination of four fungicides (azoxystrobin, chlorothalonil, cyprodinil and trifloxystrobin) residues in tea infusions was developed [69]. Under optimum conditions, the calibration graphs were linear in the range 1 – 500 µg/L. The limits of detection were (0.09, 0.18, 0.12 and 0.10 µg/L) and limits of quantification were (0.3, 0.6, 0.4 and 0.33 µg/L) for azoxystrobin, chlorothalonil, cyprodinil and trifloxystrobin, respectively.

Sol-gel technology is mainly used to synthesise sorbent materials for sample preparation techniques [53]. To this end, a highly polar sol-gel poly (ethylene glycol) (sol-gel PEG) coated fabric phase sorptive extraction media coupled with high performance liquid chromatography was applied for the determination of sulfonamides (sulfamethazine, sulfisoxazole and sulfadimethoxine) residues in milk [71]. Validation of the method was according to the European Union Decision 2002/657/EC and the decision limit values were 116.5, 114.4 and 94.7 µg/kg and detection capability were 120.4, 118.5 and 104.1 µg/kg for sulfamethazine, sulfisoxazole and sulfadimethoxine, respectively. The same FPSE media [sol-gel poly (ethylene glycol) (sol-gel PEG) coated FPSE media] was used in the extraction

of benzodiazepines most widely used as therapeutic drugs in psychiatry and most frequently encountered drugs in forensic toxicology by Samanidou et al [75], and the method produced recoveries ranging between 27 to 42 %. The same authors applied sol-gel-graphene-based fabric phase sorptive extraction coupled with high performance liquid chromatography for the analysis of bisphenol A and residual monomers including bisphenol A glycerolatedimethacrylate, urethane dimethacrylate and triethylene glycol dimethacrylate derived dental restorative materials from cow and human breast milk samples [67]. The recoveries were 50% for bisphenol A, 103% for bisphenol A glycerolatedimethacrylate, 110% for urethane dimethacrylate and 78%, for triethylene glycol dimethacrylate.

2.2.4.2 Silicone rods, tubes and discs

Silicone rods, tubes and discs are extraction methods applied to extract organic micro pollutants, similarly to SPME and SBSE with the advantage of being cheap, flexible and robust [3]. Silicone discs were used as disposable enrichment probes for the analysis of eight UV filters [2-ethylhexyl salicylate, homoalate, isoamyl-*p*-methoxycinnamate, 2-hydroxy-4-methoxybenzophenone, 3-(4-methylbenzylidene) camphor, 2-ethylhexyl-*p*-dimethylaminobenzoate, 2-ethylhexyl-*p*-methoxycinnamate and octocrylene] in surface and wastewater samples followed by organic solvent desorption, large volume injection and gas chromatography-mass spectrometry determination [76]. Linear ranges were 0.01 – 10 ng/mL for all the compounds except 2-hydroxy-4-methoxybenzophenone which was 0.04 – 10 ng/mL, with detection limits ranging from 0.003 – 0.040 ng/mL.

A monolithic porous silica disc prepared by sol-gel process then placed in an extraction chamber in the base plate of the microchip, was applied in the extraction of six proteins (insulin, cytochrome C, lysozyme, myoglobin, β-lactoglobulin and hemoglobin) with different molecular weight and isoelectric point to test the performance of the microchip [77]. High extraction recoveries of 94.8 – 99.7 % were achieved.

CHAPTER 3

3 MATERIALS AND METHODS

3.1 Chemicals

Estrone (E1), 17β-estradiol (E2) and testosterone (T) (>98% purity), were purchased from Sigma-Aldrich (Germany) and kept in a freezer (-20 °C). Merck HPLC-grade methanol, analytical reagent grade sodium chloride (NaCl) and sodium hydroxide (NaOH) salts were all purchased from (Biodynamics, Namibia). Hydrochloric acid (HCl) of analytical reagent grade was from Promark Chemicals (RSA). Ultra-pure water produced from a Millipore® Elixir Milli-Q water purifier was used to prepare all aqueous solutions.

3.2 Instrumentation

Analyses were carried out on a Thermo Scientific Focus Gas Chromatograph (GC) with a TQ-SQC capillary column (30 m × 0.25 mm) with a 5% diphenyl, 95% dimethyl polysiloxane stationary phase. The GC was coupled to a Thermo Scientific ITQ700 single quadrupole mass spectrometer (MS) detector. The GC inlet was operated in split mode (split flow set at 10 mL/min) and splitless mode (split vent closed for 4 minutes) for the method optimisation and method validation experiments, respectively. The carrier gas, helium, of 99.9% purity (Afrox, Namibia) was kept at a constant flow rate of 1.0 mL/min and passed through the Thermo Scientific (Singapore) triple air filter before it entered the GC. The injector temperature was kept at 230°C. The GC temperature program applied for method

optimisation was as follows. The oven temperature was held at 80°C for 4 min, and then increased from 80 to 300°C at 10°C/min and maintained at 300°C for 10 min. In an attempt to improve the sensitivity for the method validation experiments, the oven temperature was held at 40°C for 4 min, and then increased to 80°C at 40°C/min, after which it was increased from 80 to 300°C at 15°C/min and maintained at 300°C for 10 min. The MS ion-trap analyser was operated in full scan mode, scanning the mass range m/z 50-300 with a scan time of 0.36 sec. The MS transfer line temperature was kept at 250°C and the ion source was maintained at 200°C. For pH measurements a EUTECH Instruments (Singapore) pH meter, calibrated with certified solutions was used.

3.3 Preparation of standard solutions and spiked water samples

Individual stock solutions of E1, E2 and T were prepared at a level of 5000 µg/mL in methanol and stored at -20 °C. A series of working standards were prepared daily by diluting the stock solutions using methanol to produce solutions containing a mixture of the three steroids at various concentrations. The spiked water samples used for all the method development and validation experiments were prepared by spiking 10 ml of ultra-pure water with 50 µL of working standard solutions. Sodium chloride (NaCl), hydrochloric (HCl) and/or sodium hydroxide (NaOH) were used to adjust the ionic strength and pH values of the sample solutions, respectively.

3.4 SEP preparation and sampling procedure

SEP stalks were constructed from stainless-steel rods (90 mm × 0.66 mm) or section of a polyimide coated megabore GC column (0.530 mm I.D.). A laboratory-grade PDMS rubber tubing (PDMS sleeves) (0.64 mm I.D. × 1.19 mm O.D.) (Separation Scientific, SA) was cut into 30.0 mm pieces. The PDMS sleeves were weighed (±0.0288 g) and only the sections which were similar in weight were used. Using ethanol as a lubricant, the sleeves were slipped into position on the stalks, about 1 mm from the tip of the stalk (Fig. 3.1). Each newly used SEP was first conditioned overnight (± 18 hrs) at 230 °C in a GC injection port, operated in split mode with a nitrogen flow of 2 mL/min and a blank analysis was carried out with each new SEP to determine the efficiency of the conditioning.

Before use each day, pre-conditioned SEPs were again conditioned for 60 min at 230 °C in the GC injection port to remove any impurities. Commercially available 15 mL Supelco glass vials, with screw caps fitted with Teflon septa (Sigma-Aldrich, Germany) were used as sample bottles. The sample solution was prepared by dissolving 20 g of NaCl in 100 mL ultrapure water then adjusts to pH 7 using NaOH (0.1 M) or HCl (0.5 M) solutions. A 10 mL aliquot of the sample was placed in the sample bottle and was then spiked with 50 µL of the relevant standard solution. The SEP stalk was inserted through the middle of the inside face of the Teflon septum of a sample vial cap and moved to a position where the SEP, suspended by the cap's septum, would be directly immersed into the water sample. The cap was subsequently screwed onto the vial for extraction. The sample was agitated using a magnetic stir bar (10 mm × 4 mm) at 1500 rpm. Different extraction times and temperatures were evaluated as described in section 3.7.1. After extraction, the SEP

was removed from the sample vial cap's septum and inserted into the inside face of a GC inlet septum. The septum was moved to a position on the SEP stalk that would put the SEP (PDMS sleeve) halfway between the top and bottom of the GC injector liner. The septum containing the SEP was subsequently installed in the GC inlet in the usual way, with the SEP protruding into the inlet's liner. With the carrier gas of the GC turned off, the standard septum cap and septum was removed from the injector and the SEP was installed in the injector by inserting it into the injector septum cap with an enlarged central hole and then allowing it to drop into the injector. The septum cap was tightened, the carrier gas then turned on, and the temperature program and data acquisition was started. The SEP was left in the injector port for the duration of the analysis time. At the end of the analysis, the GC oven was allowed to cool to 40 °C and the SEP was removed and re-used for another extraction or stored in a glass culture tube. During preliminary work, carry-over experiments were conducted and results indicated that the analytes (steroids) are desorbed fully with each analysis (results not shown).

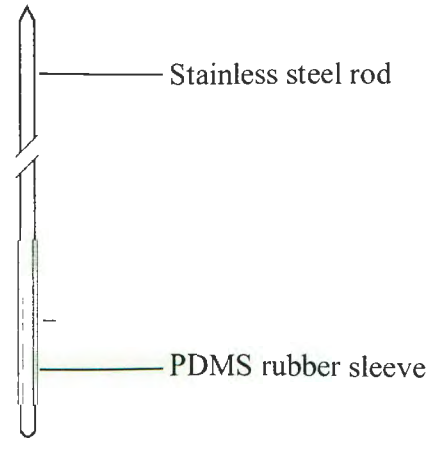


Figure 3-1: Schematic diagram of a first-generation sample enrichment probe (SEP) [10].

3.5 Method Development

The influence of various parameters on the extraction efficiency of the SEP was evaluated, namely extraction time, extraction temperature, ionic strength and pH. All the experiments were performed in triplicate. For each optimisation step the analytes response is expressed as a percentage of the highest response obtained for that set of experiments (referred to as the normalised response). Water samples spiked with the steroids at a concentration of 10 µg/mL were used for all method development experiments.

3.5.1 Optimisation of extraction time and temperature

The effects of extraction time of the target compounds on the SEP extraction efficiency were examined at 15, 30, 60 and 90 min. The temperature was kept constant at 45 °C while varying the extraction time. Subsequently, the effect of extraction temperature on the SEP efficiency was investigated, and three temperatures: 35, 45 and 55 °C were examined, while keeping extraction time constant at 60 min. The extraction time and temperature that gave optimum results were selected to be used in subsequent method development steps.

3.5.2 Optimisation of ionic strength and sample pH

Sodium chloride was added directly to 100 mL ultrapure water before spiking the sample with the steroid hormones working standard solution. A single addition of

NaCl (0.2 g/mL) was investigated. This particular NaCl concentration level was based on the optimum level determined by Yang et al [24]. The ionic strength condition that yielded optimum results for the responses of the target analytes was selected. The effect of pH on extraction efficiency was examined at pH 5 and 7 by adjusting the sample pH with HCl (0.5 M) and/or NaOH (0.1 M). The pH of the sample was measured using a pH meter. The pH level at which optimum results were obtained was selected for use in further experiments.

3.6 Method validation

The developed SEP-GC-MS method for E1, E2 and T analysis was validated in terms of its linearity, limit of quantification (LOQ), and precision. The linearity was examined using four different concentration levels, ranging from 1 to 10 µg/mL of spiked ultrapure water in triplicate. The precision of the method was assessed as its run-to-run reproducibility by analysing spiked water samples, in triplicate, at three different concentrations (0.5, 2 and 10 µg/mL) under the same conditions on the same day. The method's limit of quantitation was assigned as the lowest steroid hormone concentration that could be quantified in a sample with acceptable %RSD ($\leq 20\%$).

3.7 Method application

Water samples would be collected from four different sources; (**1**) the primary sedimentation tank (PST) of Gammams Wastewater Treatment Plant (GWWTP), (**2**) the B-series final effluent of GWWTP maturation pond, (**3**) the product water at the Old Goreangab Water Reclamation Plant (OGWRP) and from (**4**) the product water of WINGOC Water Reclamation Plant (WINGOC). Using the grab sampling method, the samples would be collected in 1L Schott glass bottles that would be first cleaned with soap, and then rinsed first with tap water followed by distilled water and chemically pure acetone. Before sampling, the bottles would be rinsed three times with the source water and then transported in an ice filled cooler box to the laboratory. To adjust the ionic strength, NaCl (20 g) would be added to 100 mL of filtered water, which would then be adjusted to pH 7 with NaOH (0.1 M) and/or HCl (0.5 M). Five 15 mL sample vials would be filled with 10 mL aliquots of the sample solution. Using a standard addition approach, the sample solutions would be spiked with different amounts (0, 50, 100, 150 and 200 µL) of the 10 µg/mL standard steroids solution. The resulting solutions would then be analysed using the validated SEP-GC-MS method as described above.

CHAPTER 4

4 RESULTS AND DISCUSSION

4.1 GC-MS analysis

Since the ITQ700 is an ion trap MS there was no improvement in sensitivity when it was operated in either selected ion monitoring mode or full scan mode. Hence all the data was acquired in the full scan mode and quantitation was performed using extracted ion chromatograms of selected ions (Table 4.1). During the preliminary steroids derivatization experiments with SEP, it was found that large amounts of bi-products were formed, presumably due to the presence of water. Since the removal of water will add a time consuming step to the method, the derivatization was omitted. The total ion chromatogram obtained using the method optimisation conditions for the analysis of a 100 µg/mL steroid standard solution is shown in Fig. 4.1. Using the optimised method, good separation of the three steroids was observed with relatively equal abundant ions.

Table 4-1: Selected ions for the quantitation of the steroids

Compound	**Retention time (min.)**	**Extracted Ions (*m/z*)**
Estrone	19.68	270
17β-Estradiol	19.78	272
Testosterone	19.92	124

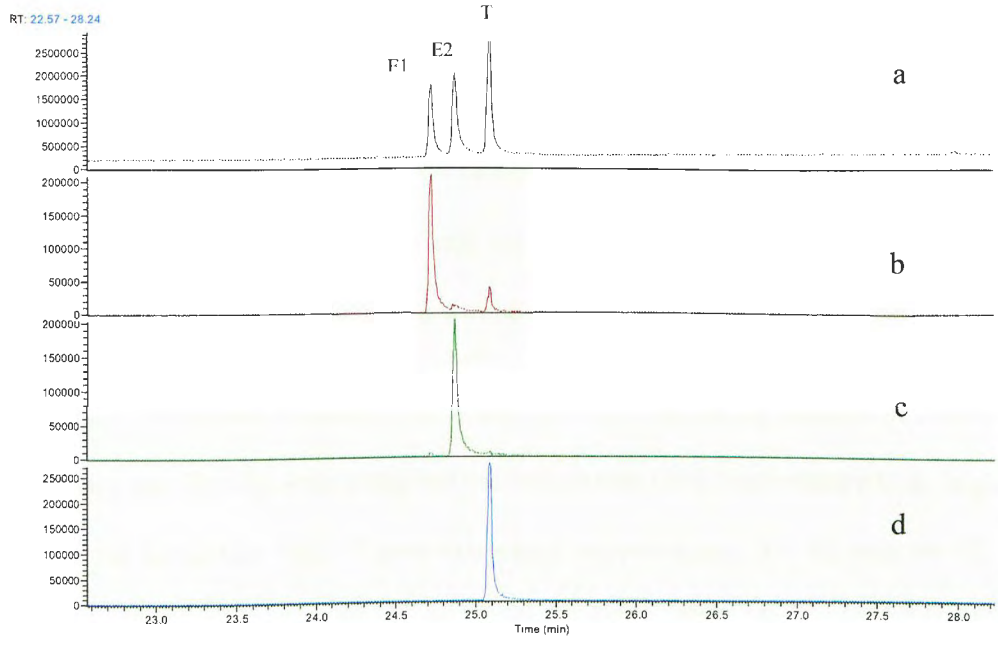


Figure 4-1: a) Total ion chromatogram of a 100 µg/mL steroids solution. Extracted ion chromatograms for b) estrone, c) estradiol and d) testosterone, respectively.

4.2 Method development

4.2.1 Optimisation of extraction time and temperature

The extraction efficiency of a large number of analyte molecules is favoured by longer extraction time to allow them to bind on active sites of the sorbent material. However, an increase in temperature allows for extraction time to be shortened as it improves the movement of the molecules, therefore extraction time and temperature are closely related [29]. Extraction efficiency depends on factors like thickness of sorbent material, agitation conditions, physico-chemical properties of the analyte and the concentration of the analyte. The distribution of analytes between the sample media and the sorbent is influenced by extraction time, thus affecting the recovery,

and it can range from minutes to hours [78]. It was observed that the responses of the target compounds increased with an increase in extraction time of up to 60 min (Fig. 4.2). However, increasing the extraction time further to 90 min resulted in a decreased response for estradiol and testosterone, while for estrone the response remained unchanged. Thus 60 min was selected as the optimum extraction time.

Since the movement of analyte molecules is improved as the extraction temperature increases and thereby increasing the extraction rate [29], temperature is an important factor for extraction [22]. Three extraction temperatures, 35, 45 and 55 °C were evaluated at a fixed extraction time of 60 min (Fig. 4.3). The results clearly shows that the responses of all the compounds increased with increasing temperatures until 45 °C, but decreased drastically when the temperature was increased to 55 °C. Based on these results, 45 °C was selected as the optimum extraction temperature. Chopra et al [15] used 55 °C as the optimum temperature for the extraction of E1 and E2 using SPME, while a temperature of 45 °C was chosen as the optimum for the same compounds [24]. However, it has been reported that lower temperatures are usually ideal in order to prevent loss of analytes adsorbed on the fibre [15].

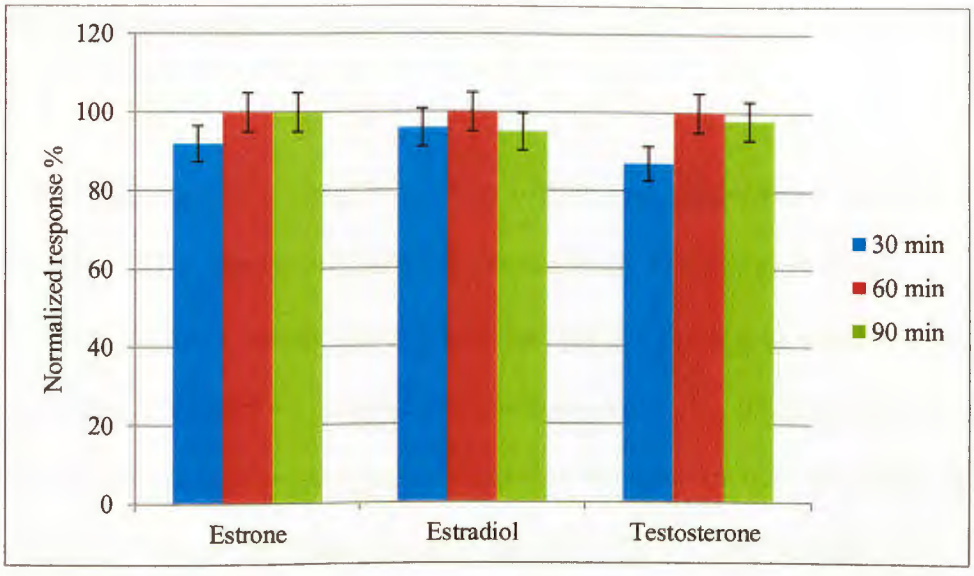


Figure 4-2: The effect of extraction time on the detector responses of the target compounds at a fixed extraction temperature (45 °C).

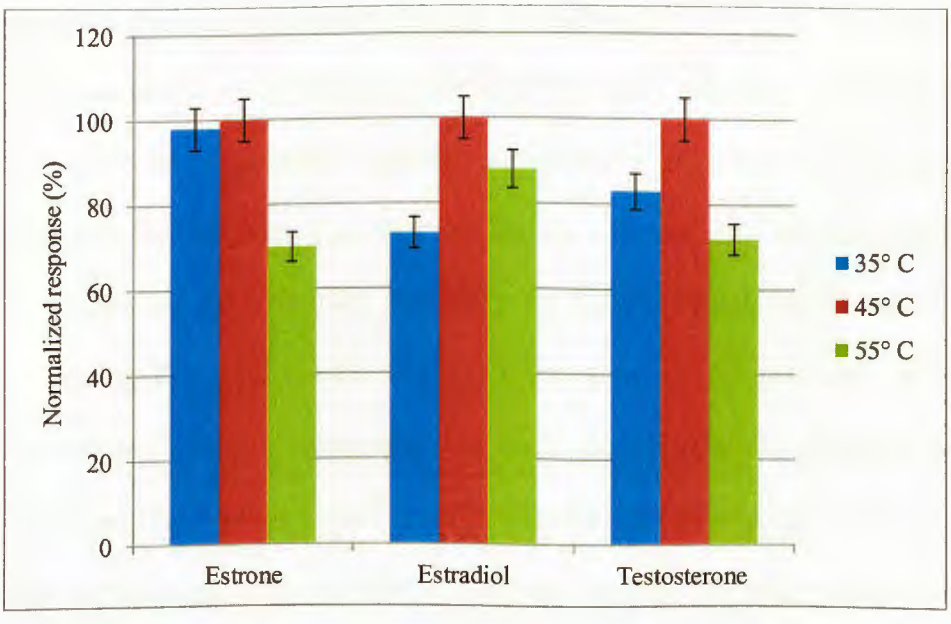


Figure 4-3: The effect of extraction temperature on the detector responses of target the compounds at a fixed extraction time (60 min).

4.2.2 Optimisation of ionic strength

After studying the effects of extraction time and temperature, the role of ionic strength of the sample solution was investigated. The addition of salt to a sample solution has been widely used to investigate the influence of ionic strength on the extraction efficiency of sorptive extraction methods [24]. The solubility of analytes decrease when salting-out agents are added to the solution, thus improving extraction efficiency of the analytes adsorbed on the PDMS [29]. Higher ionic strength decreases the solubility of the hormones in water because the water molecules forms hydration spheres around the ionic salt molecules, thus the analytes are free to adsorb onto the sorbent material [9]. The nature of the compounds investigated and the salt concentration are the two variables that determine the effect of salting-out [29]. In previous studies, an increased in the extraction efficiency of hormones as NaCl concentration levels increased (maximum extraction efficiency at 0.2 g/mL for E1, E2 and T and maximum extraction efficiency at 20% m/v for E1 and E2) was observed [24, 56]. Based on their results, the effect of ionic strength was evaluated by comparing the extraction efficiency for sample containing no salt, with those containing 0.2 g/mL NaCl. An extraction time of 60 min and an extraction temperature of 45 °C were used for these experiments. As expected the steroid hormones responses increased greatly with the increase of ionic strength (Fig. 4.4). Due to the significant increase in the responses of the target compounds in the presence 0.2 g/mL NaCl, and the fact that this was previously reported as the optimal level, the remaining experiments were performed in this way.

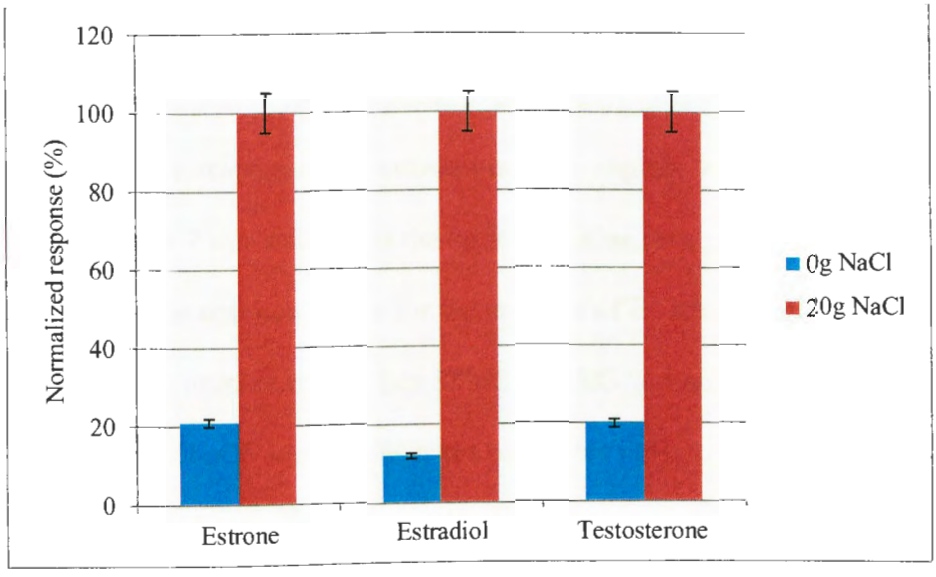


Figure 4-4: The effect of NaCl concentration on the detector responses of the target compounds at a fixed extraction time (60 min) and extraction temperature (45 °C).

4.2.3 Optimisation of sample pH

The extraction efficiencies of target compounds in a sample solution increases when the solution pH is adjusted because acids and weak bases can be converted to their neutral forms [29], ensuring that the analytes do not dissociate from the sorbent during extraction [24]. Hydrophobicity of target analytes is changed by adjusting the pH of water samples, making them easy to be adsorbed onto the stationary phase thus improving their extraction efficiencies [16]. Estrone, estradiol and testosterone have pKa values of 10.77, 10.08, and 9.74, respectively [5]. To enhance their extraction, the steroids need to be in their neutral form and this can be achieved by adjusting the sample pH [15]. Based on their pKa values it is expected that these compounds will be neutral in an aqueous solution which is at a pH of *ca.* 7. Hence the effects of the pH of the sample solution on the detector responses were examined at pH values of 5

and 7. The change in pH resulted in insignificant change on the responses of estrone and estradiol, although the optimum pH for E1 was observed at 7 and E2 at 5. However, the response for testosterone was slightly reduced at pH 5 (Fig. 4.5). Therefore pH 7 was selected as the optimum value. In a previous study a pH of 7 was selected as the optimum value for the analysis of E1 and E2 using SBSE-HPLC-UV [56], while in another study when SPME-GC-MS was applied for the analysis for E1, E2 and T, a pH of 5 was chosen as the best value [24].

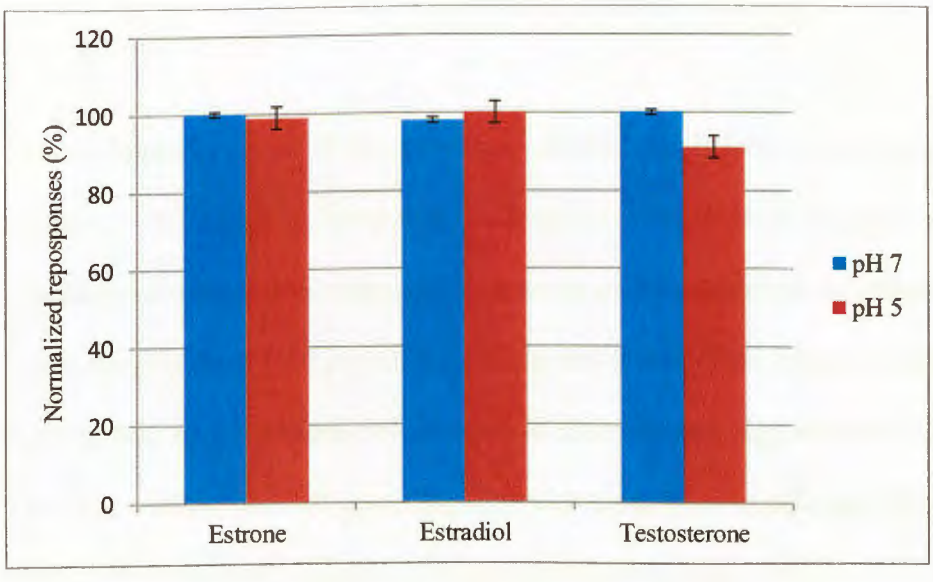


Figure 4-5: The effect of pH on the detector responses of the target compounds at a fixed extraction time (60 min), extraction temperature (45 °C) and ionic strength (0.2 g/mL NaCl).

4.3 Method validation

The analytical performance of the proposed SEP-GC-MS method for the determination of three steroid hormones in water, in terms of linear ranges, LOQs, and reproducibility were validated under optimal conditions. The summarised analytical results are presented in Tables 4.2 and 4.3.

4.3.1 Linear calibration range

The calibration curves of the developed method for the determination of steroid hormones were tested by increasing the amounts of standards in ultrapure water. The calibration standards were prepared by a series of concentrations of spiked ultrapure water, ranging from 1-10 µg/mL for estrone and testosterone, but for estradiol it was 2-10 µg/mL, as it could only be detected at concentration higher than 1 µg/mL (Fig. 4.6). The method showed good linearity with correlation coefficient (R^2) of 0.988, 0.996 and 0.992 for E1, E2 and T, respectively (Table 4.2).

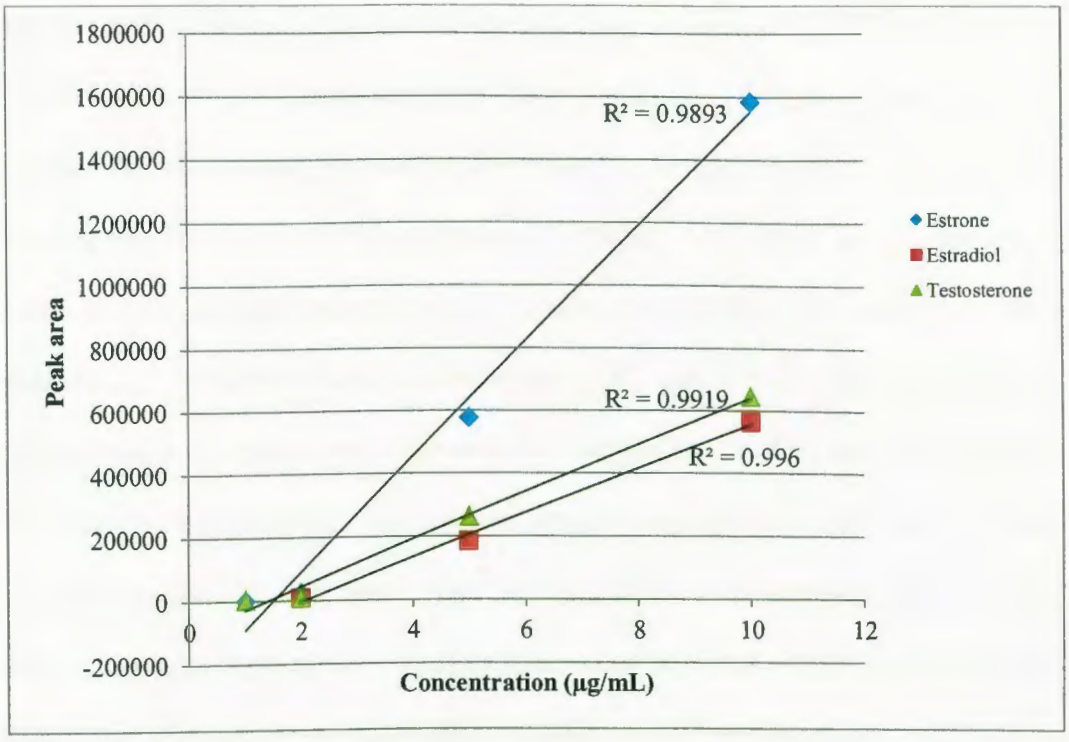


Figure 4-6: The calibration curves obtained for the steroid hormones.

Table 4-2: Linearity data obtained for the steroid hormones in spiked water samples.

Hormones	Calibration curve	R^2	Linear range (µg/mL)
Estrone	y=182500x-274344	0.989	1-10
Estradiol	y=70001x-144340	0.996	2-10
Testosterone	y=74459x-103866	0.992	1-10

4.3.2 Precision and LOQ

The precision of the developed method describes the reproducibility and expressed as %RSD was assessed by analysing spiked water samples containing known concentrations of steroid hormones, in triplicate. The %RSD were calculated from

results of the triplicate experiments at each concentration level (0.5 – 10 µg/mL) carried out under optimum conditions as presented in Table 4.3. The optimised method %RSD obtained were between 5 and 15 % for estrone, 5 and 17 % for estradiol and 4 and 9 % for testosterone. Precision (% RSD) and accuracy (% recovery) of a method should be < 20 % and between 70-120 %, respectively, for a method to be considered precise and accurate [79]. The SEP-GC-MS method for the determination of three steroid hormones in water developed in this study yielded precision (% RSD) between 5-17 percent, which are acceptable as they are not higher than 20 percent. The method's limit of quantitation was assigned as the lowest steroid hormone concentration that could be quantified in a sample with acceptable %RSD (≤ 20%) and were found to be 0.5 µg/mL for all three steroids.

Table 4-3: Reproducibility (% RSD) of the SEP method for the analysis of steroid hormones

	0.5 µg/mL	**2 µg/mL**	**10 µg/mL**
Hormones	**RSD (%)**	**RSD (%)**	**RSD (%)**
Estrone	6	5	15
Estradiol	17	6	5
Testosterone	7	4	9

Comparison of the results of the developed and validated SEP-GC-MS method obtained in this study to those reported in the literature revealed that the LOQs of the SEP method was much higher than those obtained by SPME (PA)-GC-MS [24], SPME (PDMS)-GCxGC-ToF/MS [28] and SBSE (PDMS/IRMOF-3)- HPLC-UV [56]. The lowest LOQs (0.000057-0.000393 µg/mL) were obtained when SPME (PA)-GC-MS was used for the analysis of the same three steroids, E1, E2 and T [24].

One possible reason why the latter method displayed superior sensitivity compared to the others is because the steroids were converted to trimethylsilyl ethers prior to analysis in order to improve their chromatographic peak shapes and hence their signal-to-noise ratios. However, the poor sensitivity of the SEP method reported in the current study is attributed to the sensitivity issues experienced with the GC-MS used during this study (see Section 4.4 below).

Table 4-4: A comparison of the results of the validated SEP-GC-MS with reported work.

Hormones	**Linear range (µg/mL)**	**R^2**	**LOD (µg/mL)**	**LOQ (µg/mL)**	**RSD (%)**	**Analysis method**	**References**
Estrone	1-10	0.989	0.5	1	5-15	SEP-GC-MS	Current method
Estradiol	2-10	0.996	2	2	5-17		
Testosterone	1-10	0.992	0.5	1	4-9		
Estrone	0.0001-0.1	0.999	0.000007	0.000057	8	SPME-GC-MS	[24]
Estradiol	0.0001-0.1	0.996	0.000007	0.000022	10		
Testosterone	0.001-1	0.999	0.000017	0.000393	8		
Estrone	0.0012-.012	0.970	0.00002	0.0012	-	SPME-GCxGC-ToF/MS	[28]
Estradiol	0.0021-0.05	0.970	0.00134	0.0021	-		
Estrone	0.001-2.5	0.991	0.00029	-	5.8	SBSE-HPLC-UV	[56]
Estradiol	0.001-2.5	0.997	0.00028	-	4.5		

4.4 Challenges during method development and validation

One of the challenge that was experienced for the duration of this study was bleeding of material from the SEPs which often caused interferences (results not shown) as well as contamination of the MS source which consequently also affected the sensitivity of the MS. This was particularly pronounced once repeated experiments with the same SEP were being performed with the addition of NaCl to the water samples and it affected the reproducibility as well as the sensitivity of the method. It was noticed that at the same time the stainless steel stalks of the SEPs were rusting, hence the remaining experiments were performed using sections of a polyimide coated megabore GC column as stalks to construct the SEPs. The method optimization experiments were performed with stainless steel stalk SEPs while the method validation experiments were performed with polyimide coated megabore GC column stalk SEPs. However, the extremely poor sensitivity of the final method is ascribed to the fact that the GC-MS instrument was not functioning properly at the time that the method validation experiments were performed. Due to these challenges encountered with the GC-MS experiments with wider concentration range and recoveries could not be performed. Unfortunately the instrument could not be repaired in time in order to repeat the validation experiments, thus the method was only partially validated.

4.5 Method application

It was envisaged that water samples from four different sources; (**1**) the primary sedimentation tank (PST) of Gammams Wastewater Treatment Plant (GWWTP), (**2**) the B-series final effluent of GWWTP maturation pond, (**3**) the product water at the Old Goreangab Water Reclamation Plant (OGWRP) and from (**4**) the product water of WINGOC Water Reclamation Plant (WINGOC) would be analysed using the validated SEP-GC-MS method. Quantitation of the steroids in the water samples would have been performed using a standard addition approach as described in Chapter 3 of this thesis. Unfortunately the GC-MS was out of order at the time that the study reached the point of method application. Therefore these experiments could not be carried out in time.

CHAPTER 5

5 CONCLUSIONS

This is the first application of the SEP coupled to GC-MS for the quantification of three selected steroid hormones, estrone, estradiol and testosterone, in water. A simple SEP-GC-MS method was successfully optimised and validated. The optimised extraction time, extraction temperature, salt concentration and pH of the SEP method were 60 min, 45 °C, 20 g/mL NaCl and pH 7, respectively. In particular, the increase of the ionic strength of the water samples by the addition of salt resulted in a significant improvement of the recovery efficiency of the SEP method for all three steroid hormones. The developed method was validated and showed good linear in the ranges of 1-10 µg/mL (E1 and T) and 2-10 µg/mL (E2) with good precision. However, in comparison to the existing SPME and SBSE extraction methods for estrone, estradiol and testosterone determination, the current method gave very high LOQs. On the other hand, the method proposed herein is simple, cost-effective and environmental friendly. In addition, if the developed SEP method could be validated on a high sensitivity GC-MS instrument it should produce results comparable to those of SPME and SBSE methods.

RECOMMENDATIONS

Only SEPs with a section of a polyimide coated megabore GC column stalks should be used in future since the stainless steel stalks may start rusting after repeated use. The SEPs should also be subjected to a more rigorous conditioning process in order to avoid interferences and MS source contamination due to SEP bleed. The sensitivity of the GC-MS was very poor at the time that the validation experiments were performed as it was not functioning properly. Therefore, validation experiments should be repeated once the GC-MS is functioning at its full potential. The determination of LODs can then also be performed. Thereafter, the validated method should be used for the analysis of water samples from the relevant water sources as described.

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